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Utilization of horseradish (*Moringa oleifera*) as an alternative protein-source feed ingredient on the diet of red Nile tilapia (*Oreochromis niloticus*)

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Abstract
Reduced feed cost is very much desired in aquaculture and usually this is done by reducing the level or substituting expensive feed ingredients with cheaper ones without detrimental effects on the growth and/or health of the animal. *Moringa oleifera* is one of the well-studied plants that contains necessary nutrients and have a great function in providing health and medicinal benefits. Concern relating to biosecurity and food safety have enhance the consumers awareness in health concerns related to chemical contaminants and this alter the consumer eating patterns and has stimulated production of organically-raised foods. The study was conducted to evaluate the nutritive value of formulated diet with *M. oleifera* leafmeal and determine the optimum dietary level of *M. oleifera* that would provide better growth and survival to red Nile tilapia. The treatments evaluated were incorporation of 0, 10%, 15% and 20% levels of *M. oleifera* leafmeal on the diet of red Nile tilapia. Proximate analysis revealed that experimental diets contained levels of crude protein ranging from 29.84% to 31.63%. Results of the study showed that fish fed with diets containing *M. oleifera* leaf meal resulted in comparable growth and survival with those fed the control diet. Fish fed with experimental diet of 20% *M. oleifera* performed better than those fed with diets containing 10% and 15% *M. oleifera* leaf meal. The study concluded that *M. oleifera* can potentially use as an alternative protein-source feed ingredient. Further, inclusion of up to 20% *M. oleifera* in the diet of red Nile tilapia can provide acceptable growth performance.

Keywords: Moringa, horseradish, red tilapia, proximate analysis, growth performance

Introduction
Horseradish grow in many tropical and subtropical countries under stressful environmental conditions and requires lower external energy (Sanchez-Machado *et al*., 2010) [26]. *Moringa oleifera* is one such plant, which has been reported to possess many medicinal properties (Dewangan *et al*., 2010) [5]. Almost all parts of the *M. oleifera* tree are edible and medicinal and have long been consumed by humans as early as ancient Roman and Greek times (Magat *et al*., 2009) [17]. The leaves and pods are used as food supplements, as potential source of protein and as an excellent source of vitamins, minerals and amino acids for humans and animals (Makkar and Becker, 1996) [18]. Nile tilapia have become an excellent choice for aquaculture (El-Sayed, 2006) [6]. Positive aquacultural characteristics of tilapia includes their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms (Popma and Masser, 1999) [22]. The presence of red varieties of *Oreochromis niloticus* (Linnaeus), *O. mossambicus* and red hybrids, such as the Florida red tilapia (FRT), Taiwanese red tilapia and the Philippine red tilapia, has also been commercially important in the cultivation of these cichlids in aquaculture (Behrends *et al*., 1982; Wohlfarth *et al*., 1990; Hulata *et al*., 1995 and Majumdar *et al*., 1997) [3, 31, 13, 19]. In Mexico, red tilapia is preferred over wild-type tilapia, and the price for red ones is usually higher than that for wild types. In some Asian region, the red tilapia (Oreochromis spp.) is the dominant species account for up to 85% culture status particularly in Malaysia. (Ng and Hanim, 2007) [20]. Consumers have shifted on red tilapia in their preference for food in contrast with the regular silvery/olive colored tilapia due to its colouration and appearance. In addition, aquaculturist project higher return of investment upon culturing red tilapia because it fetches a higher price (Ng and Hanim, 2007) [20]. In aquaculture systems, the increasing price of feed due to expensive feed ingredient is considered one of the most important factors that limit profitability (Tagwireyi *et al*., 2008) [29].

Thus, studies and researches are essential to develop economically viable feeds to further attain desirable return of investment among fish culturist (Lunger *et al.*, 2006) [16]. The study aimed to determine the nutritive value of formulated diet with *M. oleifera* leafmeal and evaluate the effectiveness of *M. oleifera* as an alternative protein-source feed ingredient in promoting growth and survival to red Nile tilapia. It also aimed to determine the optimum level of *M. oleifera* upon inclusion in the fish diet.

Materials and Method

Formulation of experimental diets

The matured leaves of *M. oleifera* were oven-dried at of 40 ºC for four hours. The oven dried leaves were pulverized into fine powder using pulverizer. Four diets were formulated and served as treatments of the study: Treatment I – control diet (0% *M. oleifera* leaf meal); Treatment II - diet with 10% *M. oleifera* leaf meal; Treatment III - diet with 15% *M. oleifera* leaf meal and Treatment IV – diet with 20% *M. oleifera* leaf meal. Feed formulation is described in Table 1 which are composed of fish meal, rice bran, soybean meal, vitamin-mineral mixture, dicalphosphate, oil and *M. oleifera* leaves.

Table 1: Components of the experimental diets (%)

Feed Ingredients	Treatments			
	I	II	III	IV
M. oleifera leaf meal	0	10.00	15.00	20.00
Fishmeal	18.00	18.00	18.00	18.00
Rice bran	46.20	39.10	35.70	32.20
Soybean meal	31.80	28.90	27.30	25.80
Vit-mineral mixture	2.00	2.00	2.00	2.00
Dicalphosphate	1.00	1.00	1.00	1.00
Oil	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

Experimental Fish and Set-up

Red Nile tilapia fingerlings with initial weight ranging from 3 to 5 grams were used in the study. The fingerlings were obtained from the Freshwater Aquaculture Center, Central Luzon State University. Before the start of the experiment, the fingerlings were acclimatized for 14 days and starved within 24 hours. Twelve indoor glass aquaria with 50 L capacity were used in the study. The fish were randomly distributed into the aquaria at a stocking density of 20 fingerlings per aquarium. Aeration and daily cleaning of aquaria and changing of water were administered to maintain good water environment for the fish. Feeding was done thrice a day at 8:00 am, 11:00 am and 3:00 pm. The ration was adjusted every two weeks where new mean weights of fish were determined through sampling. Ten percent feeding rate was used and reduced to eight percent after 45 days. The study was administered for a total of 12 weeks culture period.

Proximate analysis of experimental diets

Proximate analysis of the experimental diet was conducted at the Nutrition Laboratory of Freshwater Aquaculture Center, Central Luzon State University. The crude protein content was determined using the Kjeldahl method (N x 6.25). The crude lipid content was determined using the Goldfisch method by extraction with petroleum ether. The moisture content was determined using a drying oven at 40 degree Celsius for six hours. Ash was determined by incineration of a pre-weighed sample in a silica crucible in a muffle furnace at 600 degree Celsius for three hours. Crude fiber in feeds was obtained by the acid alkaline method.

Statistical Analysis

Differences in initial weight, final weight, final length, body weight gain, specific growth rate, absolute growth rate and survival rate were analyzed using one-way analysis of variance (ANOVA). Comparison of means was done using Duncan Multiple Range Test at significant level of ($P<0.05$).

Results and Discussion

Growth parameters attained common outcome in which all treatments revealed to have no significant difference ($P<0.05$) in terms of final weight, body weight gain, specific growth rate and absolute growth rate. This indicated that comparable growth performance was attained between the control diet and the diets with *M. oleifera* leafmeal. However, among treatments incorporated with *M. oleifera* leafmeal, the diet with 20% level of incorporation had the highest result on the growth of red Nile tilapia. It was also notable that there was a decreasing trend of growth as the level of incorporation decreases from 15% up to 10% level of incorporation. This signified that addition of *M. oleifera* leafmeal up to 20% in the diet of red Nile tilapia will provide acceptable growth performance. Result in terms of survival rate revealed that all treatments were insignificantly different ($P<0.05$) with each other. However based on values, diet without incorporated *M. oleifera* leafmeal had the lowest survival rate. Table 2 shows the growth performance of red tilapia in terms of initial weight, final weight, final standard length, body weight gain, specific growth rate, absolute growth rate and survival rate after 12 weeks of culture.

Table 2: Growth performance (± standard deviation) of red tilapia after 12 weeks of rearing

Parameters	Treatment	Treatment	Treatment	Treatment
	I	II	III	IV
Initial weight (g)	3.89[a] (±0.189)	3.74[a] (±0.119)	3.91[a] (±0.106)	3.76[a] (±0.006)
Final weight (g)	23.12[a] (±1.196)	17.71[a] (±1.609)	18.94[a] (±1.617)	21.83[a] (±1.867)
Final standard length (cm)	8.76[a] (±0.163)	8.45[a] (±0.349)	8.4[a] (±0.274)	8.8[a] (±0.167)
Body weight gain (g)	19.23[a] (±1.303)	13.98[a] (±1.72)	15.04[a] (±1.513)	18.08[a] (±1.074)
Specific growth rate (%)	1.82[a] (±0.087)	1.58[a] (±0.128)	1.6[a] (±0.057)	1.79[a] (±0.048)
Absolute growth rate (g/day)	0.2[a] (±0.023)	0.14[a] (±0.03)	0.15[a] (±0.027)	0.19[a] (±0.019)
Survival rate (%)	83.33[a] (±14.240)	88.33[a] (±1.667)	91.67[a] (±4.410)	86.67[a] (±1.667)
Means in rows with similar superscript are not significantly different at 5% level of significance.				

Proximate analysis (Table 3) revealed that experimental diets contained levels of crude protein ranging from 29.84% to 31.63%. In addition, diets with *M. oleifera* leaf meal had slightly high analyzed crude protein contents than the control diet. However, the higher levels of analyzed crude protein in the diets with *M. oleifera* leaf meal simply resulted to comparable growth of the fish with the fish fed with control diet.

Table 3: Proximate analysis conducted on the experimental diets

Nutrients (%)	Treatment I	Treatment II	Treatment III	Treatment IV
Crude protein	29.84	30.84	30.52	31.63
Crude fat	1.87	1.62	1.66	1.42
Crude fiber	15.33	11.62	10.46	10
Moisture content	13.11	11.95	11.78	12.36
Dry matter	86.89	88.05	88.22	87.64
Mineral matter or ash	18.05	17.05	16.58	16.61

Afuang *et al.* (2003) [2] reported that solvent– extracted *M. oleifera* leaf meal could replace up to 30% of fish meal from *Oreochromis niloticus* diets. On the other hand, result of Francis *et al*. (2002) [9] experiment using *M. oleifera* leaf meal in *Oreochromis niloticus* diets showed growth reducing effects at high levels of inclusion of raw leaf meal. Furthermore, the replacement of fish meal in a diet for tilapia with *M. oleifera* leaf meal beyond a 10% level produced poor growth and lowered the feed utilization efficiency (Richter *et al.*, 2003) [23]. Results of this study, however, revealed that *M. oleifera* leaf meal at high level of incorporation (20%) gave the best result on growth performance of red tilapia compared to lower levels (10% and 15% of the diet). Protein is very important in fish growth and thus crucial ingredient in fish diets (Tagwireyi *et al.*, 2008) [29]. In order to reduce feed cost used in aquaculture identifying alternative protein sources is necessary in which *M. oleifera* is one of the experimented promising plant protein sources for aquaculture (Abo-State *et al.*, 2014) [1]. *M. oleifera* leaf protein concentrates contained high amount of crude protein. The presence of significant quantity of crude protein in Moringa means that Moringa leaf protein concentrates could be used as nutritionally valuable healthy ingredient to improve protein deficiency of fish (Sodamade *et al*., 2013) [28]. The result of the study concluded that due to the high level of protein in *M. oleifera* a better performance is noticeable to the experimental treatment which has the highest level of *M. oleifera* leaves.

Heat treatment methods employed in the preparation of *M. oleifera* leaves might have increased the digestibility of proteins and other dietary components such as starch related compounds and might reduce palatability-reducing factors (Tagwireyi *et al.*, 2008) [29]. This address the concern in connection to the digestibility and palatability of *M. oleifera* leaves where oven drying is involved on the preparation that may have contributed to the acceptable utilization of fishes. The reduction in anti-nutrients by processing techniques such as soaking, drying and heat treatment on plant-based fish ingredients have resulted in better palatability, increased feed digestibility and growth in fish (Sidduraju and Becker, 2003; Rweyemamu, 2005) [27, 25]. Organic production of herbivorous and omnivorous aquaculture species appears to be relatively straight forward as organic feedstuff may largely cover their nutritional needs and therefore readily replace conventional feedstuff (Craig and Mclean, 2005; Li *et al*., 2006) [4, 15]. Much of the current research efforts in conventional feed for cultured species examines the substitution of fish meal by vegetable proteins (Gatlin *et al*., 2007; Glencross *et al*., 2010; Øverland *et al*., 2009) [10, 11, 21]. and studies have shown that it is possible to substitute a significant part of fish meal with plant protein concentrates without compromising fish growth when supplementing the diet with indispensable amino acids (Kaushik *et al*., 1995; Rodehutscord *et al*., 1995) [14, 24]. An issue of more recent concern relates to that of biosecurity and food safety that consumers have enhanced education and awareness in health concerns related to chemical contaminants (Hites *et al*., 2004) [12]. This alter the consumer eating patterns has stimulated production of organic foods. Consumers prefer organically produced food because of perceived health attributes and concern about pesticide residues, the environment, and farm worker safety (USDA Economic Research Service, 2003). The principles of organic aquaculture encourage the development of fish feeds containing fish meal from sustainable fisheries only to avoid depleting global fish stocks and stresses the need for alternative, organic feed ingredients (EU, 2007, 2009) [7].

In the current study, the result recommended an inclusion up to 20% of *M. oleifera* leafmeal promote acceptable growth performance and survival in red Nile Tilapia. This outcome may provide venue of adaptation among fish farmers and feed manufacturers to utilized feed ingredient having a healthy outlook profile to consumer besides its prime benefit of reducing the feed cost. It is not negligible that utilization of *M. oleifera* as a feed ingredient is considerably an effort towards the attainment of organically raised aquaculture species.

Conclusions

The study concluded that *M. oleifera* can potentially use as an alternative protein-source feed ingredient on the diet of red Nile tilapia. Further, utilization of up to 20% level of incorporation of *M. oleifera* leaf meal can provide acceptable growth performance and survival to red Nile tilapia.

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